Screening methods for waterlogging tolerance in *Urochloa* grasses

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Highlights

- Waterlogging is a stress experienced by the roots and reflected upon the shoot. As such, screening procedures that includes root traits that are known to confer adaptation to waterlogging may greatly improve selection for waterlogging tolerance in *Urochloa* grasses.
- Screening procedures should be performed under adequate nutrient levels to avoid confusion of low growth rates and waterlogging tolerance.
- Conatiner size have an effect upon waterlogging tolerance. A long cylinder system is recommended to screen rooting depth (an proxy for root aeration efficiency in *Urochloa* grasses) under waterlogged soil conditions.

Background

Waterlogging is a major environmental stress that affects production and persistence of *Urochloa* pastures. Since 2007, CIAT (now the Alliance of Bioversity International and CIAT, ABC) and partners have used different screening methods based on morphological and physiological attributes to identify *Urochloa* genotypes that are tolerant to waterlogging. Over the years, research carried at ABC staff (past and present) has identified several responses of *Urochloa* grasses under waterlogging conditions. This has allowed the identification of mechanisms and traits behind such responses. This body of research can be accessed in the links below:

(year, topic, link)
2007, General responses at shoot level, https://bit.ly/3oAjcFK
2013, Root metabolic responses, https://bit.ly/3kONN1u
2013, Morpho-anatomical responses of shoot and roots, https://bit.ly/3qONFTm
2014, Changes in root architecture, https://bit.ly/30Fm78l
2015, Effect of nutrient content, https://bit.ly/3FuT0TS
2015, Antioxidant responses/ROS scavenging, https://bit.ly/3DxLg2T
2019, Effect of nutrient content in root anatomy, https://bit.ly/3DvBJcE
2019, Apoplastic exclusion of Fe from roots, https://bit.ly/323Qd5F

Albeit complex, this body of research indicate the following. *Urochloa* grasses acclimate to short term waterlogging (< 3 days) by increasing alcohol dehydrogenase activities in their roots. However, the main mechanism of long-term adaptation (> 3 days) involves an improved aeration system from shoot to root (aerenchymatous tissue). An improved aeration system allows the maintenance of aerobic root respiration in a hypoxic-anoxic environment such as waterlogged soil. Better adapted genotypes have faster development of nodal roots that replace a decaying old root system due to waterlogging. These newer roots have greater extent of aerenchyma and a stronger barrier of radial oxygen loss in the outer part of the roots. Nutrient content (including microelements) has an influence of waterlogging tolerance. Plants grown under low nutrient content (soil or stagnant nutrient solution that mimics waterlogging) appeared more tolerant to waterlogging; greater concentration of redoximorphic microelements (Fe and Mn) results in plants with greater damage due to waterlogging (e.g., stunted growth, increased leaf senescence). The most conspicuous and easiest of traits to record among *Urochloa* grasses, to differentiate their waterlogging tolerance, probably are a) at the shoot level, leaf senescence, and b) at root level, rooting depth. As a result of this body of research, several screening methods have been developed over the years

(year, topic, link)
2007 General responses at shoot level https://bit.ly/3Cqe389
2013 General screening methods https://bit.ly/3kOjCHH
2017 Image based-field screening methods https://bit.ly/3coHZae
2018 Greenhouse based screening method https://bit.ly/3HAih12

Effect of container size upon waterlogging tolerance in *Urochloa* grasses

Rationale

One aspect that has somehow lost in the process of developing reliable screening procedures, is to test the effect of container size upon waterlogging tolerance. Considerable research has shown that container size can have a limiting effect on plant growth. This research aimed to help to fill this gap and examined the effects of pot size (pots of 1.75kg, 3.5 kg and a cylinder of 4.0 kg) on biomass production of contrasting *Urochloa* genotypes (the tolerant *U. humidicola* CIAT 679; the moderately tolerant *U. brizantha* CIAT 26110; and the sensitive *U. ruzizinesis B*r 44-02) under two soil conditions: drained (80-10 field capacity) and waterlogged for 21 days. This will in turn aid to establish more efficient and reliable screening procedures for waterlogging tolerance needed to assist the *Urochloa* breeding program at CIAT.

Materials and methods

One trial was conducted outside in the Forages patio area of ABC, Palmira to determine the influence of container size in three *Urochloa* genotypes with differential tolerance to soil waterlogging (CIAT 679, CIAT 26110 and Br 44-02).
Three types of containers were used: a) pots of 15cm of height x 15cm diameter, filled with 1.75Kg of soil; b) pots of 20cm of height and 30cm of diameter filled with 3.5 of soil and c) transparent cylinders (80cm tall x 7.5cm diameter) filled with 4.0kg of soil inserted into a PVC pipe of the same dimensions). Soil was a top Oxisol (0-20cm) from Santander de Quilichao. An adequate amount of fertilizer was supplied (kg ha^{-1}: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo) to soil at the time of planting. Containers were arranged in a split plot design with four replications per genotype and treatment. Two vegetative propagules were sown and after 5 days of rooting, thinned to one. Three weeks after rooting, two treatments were imposed: draining (80-100% field capacity) and waterlogging. Start of experiment was based on observation of root development of plants grown in transparent cylinders (approximately 20cm of root depth). Waterlogging was imposed by applying excess water and maintaining a 3cm water lamina over the soil for 21 days. In waterlogged pots, drainage of water was prevented with an exterior pot of slightly higher size that it was covered by a plastic bag. This second pot acted as a bucket that prevented drainage. In waterlogged cylinders, drainage was avoided by putting a tap in the bottom of the PVC pipe.

After 21 days of treatment, shoot was separated from roots. Shoot was further separated into leaves, stem and dead leaves and oven dried at 70ºC for 3 days. Roots were washed free of soil and kept in 50% ethanol solution and kept at 4ºC for posterior determination of root porosities. Roots of waterlogged plants were further separated, and dead roots were manually separated. Live roots have a white and turgid appearance, in contrast to dead roots that appear dark and deflated.

Root porosities

Porosity was determined using the pycnometer method which estimates volume occupied by air in roots. A sample of 4 to 5 roots in each container was taken, and each cut into 2-3cm segments. Porosity was calculated as % = 100 x (P_G – P_R)/ (P + R + P_R), where R is the fresh mass of intact roots, P_R is the pycnometer mass with water and intact roots, and P_G is the pycnometer mass with water and roots previously macerated with a mortar. Once porosities were recorded, root dry biomass of nodal and lateral roots was determined.

Percentage of change of tissue mass fractions

Green leaf biomass proportion (GLBP = 100 x green leaf biomass/ total leaf biomass); leaf mass ratio (LMR = 100 x green leaf biomass/total biomass), stem mass ratio (SMR = 100 x stem biomass/total biomass x100); root mass ratio (RMR = 100 x root biomass/total biomass) and dead leaf biomass ratio (DLR = 100 x dead leaf biomass/total biomass) and the changes of ratios of waterlogged plants relative to drained plants were calculated (% Change).

Results and discussion.

Waterlogging induced an increment in porosities of roots in all containers of different size. High root porosity is an indication of aerenchyma. A preliminary study showed a positive correlation (r = 0.8 ***) between aerenchyma and root porosity in *Urochloa* genotypes when porosity was above 10% (i.e., porosity values under 10% arose mainly from intercellular spaces in the root cortex rather than aerenchyma presence). It is well known the functional role of aerenchyma for adaptation to waterlogged conditions, allowing oxygen transport and thus root functioning in hypoxic-anoxic environments. Under waterlogged conditions, the tolerant CIAT 679 showed higher values of root porosities (>30%) than CIAT 26110 (<19%) and Br 44-02 (<21.5). All plants of CIAT 679 showed higher values of porosities under drained conditions (≤20). The presence of high porosities under drained conditions in CIAT 679 supports the notion of constitutive aerenchyma in this genotype. Constitutive aerenchyma is common in plants adapted to oxygen shortage, and would represent an advantage at the first stages of waterlogging as aerenchyma takes time to form. Notwithstanding, the less adapted genotypes to waterlogging, CIAT 26110 and Br 44-02, also showed high porosities (>15%) when grown in pots (1.75kg, and 3.5 kg), but very low porosities (below 5%) under drained conditions. High porosities of CIAT 26110 and Br 44-02 under drained conditions in pots, but not in cylinders, suggests that aerenchyma development was influenced by the restriction of root penetration in pots of 1.75 kg (15 cm of height) and 3.5 kg (20 cm of height). Root aerenchyma might be a key trait to select in a plant breeding program for waterlogging tolerance. However, as shown in this study, the screening for aerenchyma in *Urochloa* genotypes using pots would confound effects and may introduce artifacts, such as the wrong conclusion of constitutive aerenchyma in least adapted genotypes.

Waterlogging induced a reduction of root biomass in all genotypes but container size influenced the amount of reduction (Figure 5). Plants grown in waterlogged pots of 1.75kg showed less reduction of root biomass (Figure 4). Root depth is highly dependent of transported oxygen via aerenchyma under waterlogged conditions. Root depth at harvest for waterlogged plants of CIAT 679, CIAT 26110 and Br 44-02 was approximately 25cm, 10, and 11cm respectively (Data not shown). The largest reduction of roots biomass of plants grown in waterlogged cylinders was due to the restriction of root growth deeper down the soil. On the other hand, root weight was less reduced on the smaller pot size maybe due to the ability of plants to produce enough aerenchyma to sustain root growth in the upper layers of waterlogged soil. This means that smaller pots, with smaller heights may obscure the negative effect of waterlogging on root growth.

Green leaf biomass proportion has been used as a tool for identification of genotypes with superior waterlogging tolerance in *Urochloa.* The present study has showed that GLBP of waterlogged plants grown in different containers remained relatively unchanged (Figure 7). However, when comparing to the drained containers, differences were more marked for plants grown in cylinders than for pots of 3.5kg, and pots of 1.75kg. The present study showed that waterlogging induced reduction of roots, leaves and stems in all genotypes (Figures 2 to 5) in all genotypes and an increase in dead leaf biomass in CIAT 26110 and Br 44-02 (Figure 6). However, the type of container had an influence on plant growth and smaller pots showed a less marked differences both when comparing responses among genotypes but also when comparing the relative changes compared to the drained treatment. (Table 1).

Root depth is restricted during waterlogging and it is related to the percentage of aerenchyma in roots. Root growth is even restricted for waterlogging tolerant genotypes such as *U. humidicola* and rarely grow deeper than 30cm. Calculations of maximum root length of several *Urochloa* genotypes in waterlogged soil, based on O_2 diffusion via aerenchyma, predict root lengths between 15 and 35 cm (data not shown). The results suggest that plants grown in long cylinders could serve to screen for differences in root depth and development under waterlogged conditions.

Figure 1. Root porosity of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

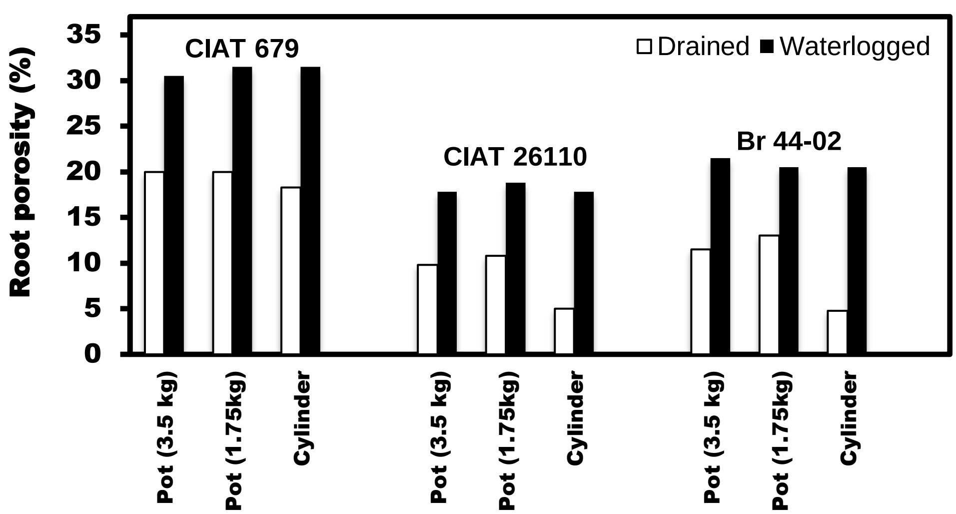

Figure 2. Foliar area of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

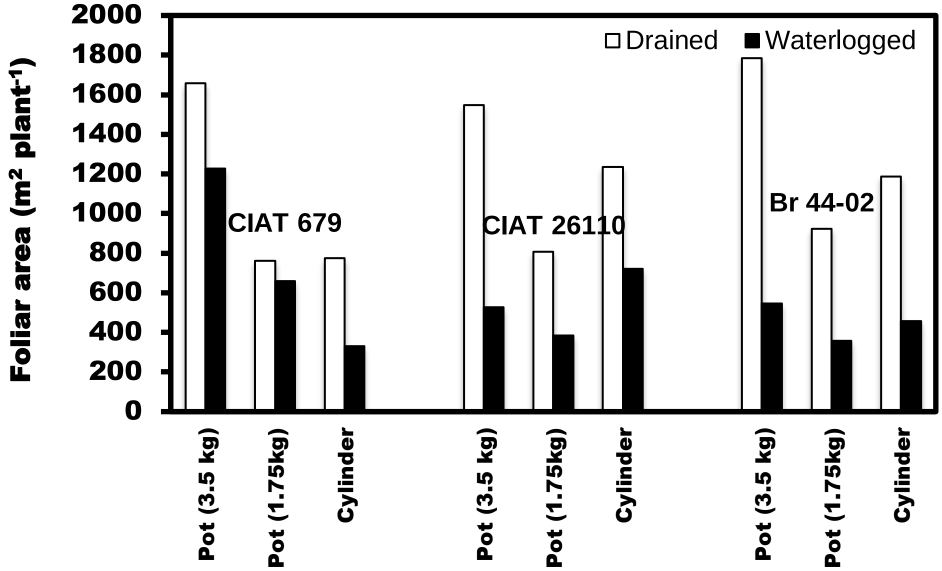

Figure 3. Stem dry biomass of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

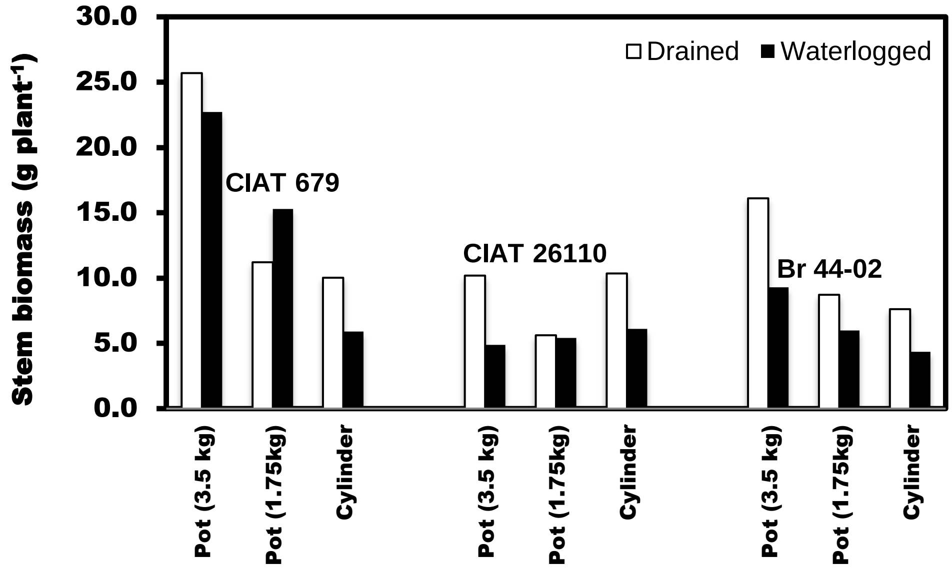

Figure 4. Green leaf dry biomass of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

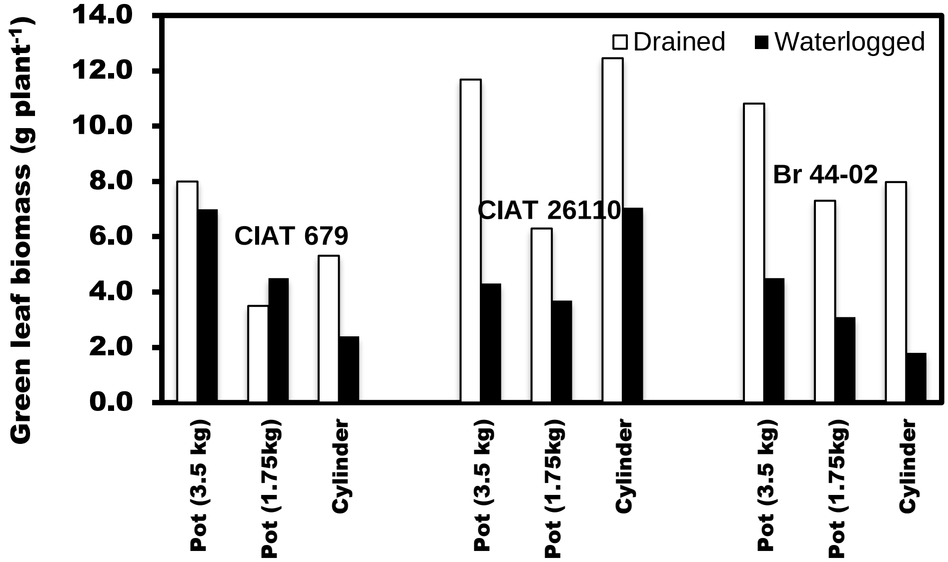

Figure 5. Root dry biomass of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

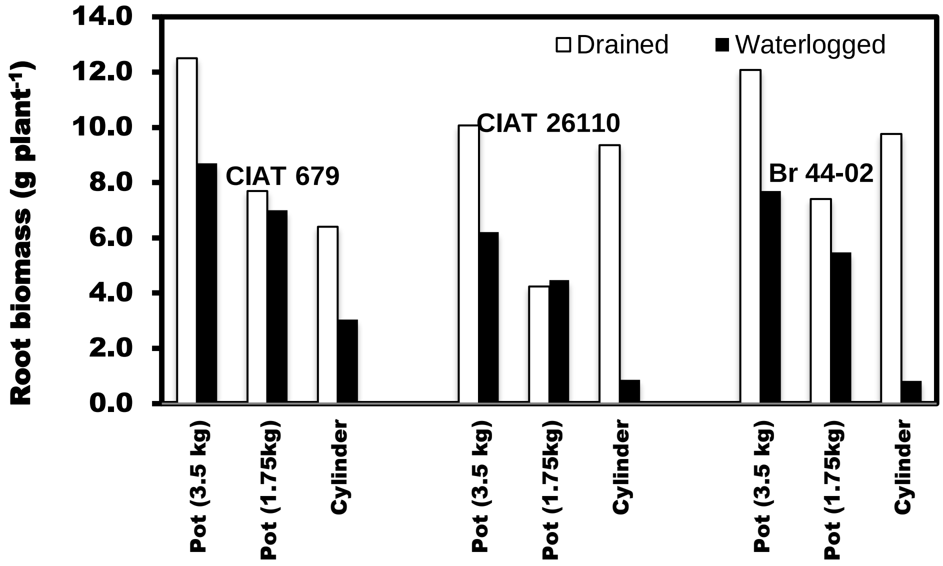

Figure 6. Dead leaf dry biomass of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

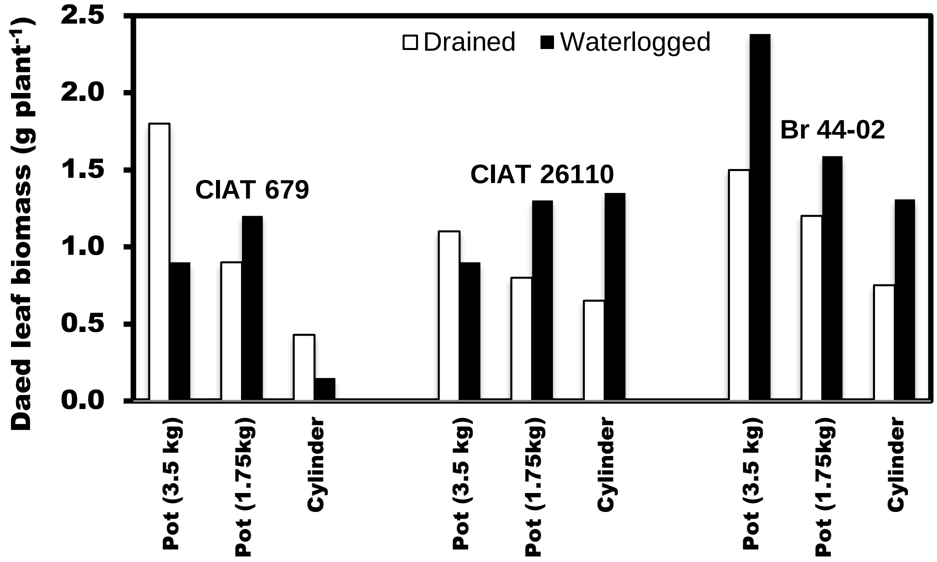

Figure 7. Green leaf biomass proportion (GLBP%) of plants grown in different containers and subjected to drained or waterlogged soil conditions for 21 days.

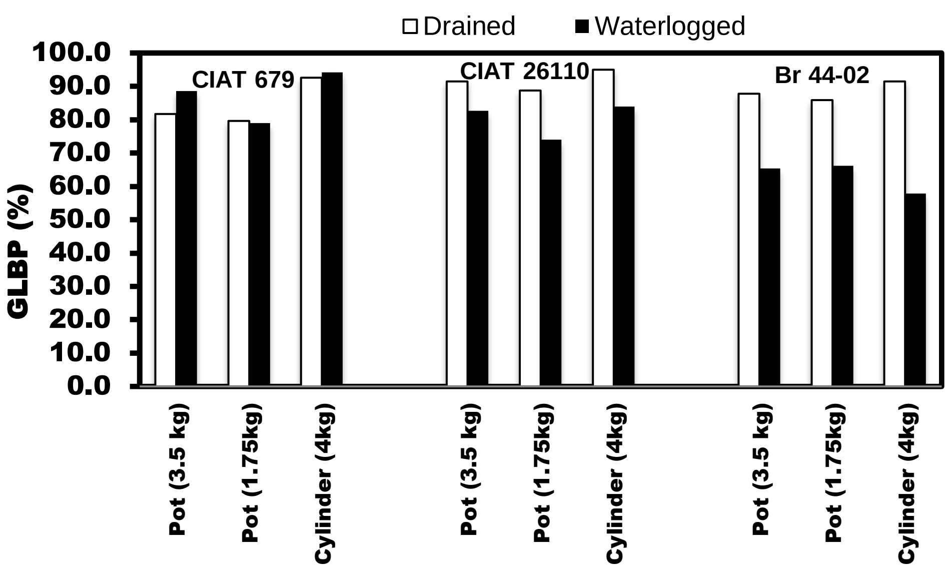

Table 1. Percentage of change of dry mass ratios. Green leaf biomass proportion (GLBP); leaf mass ratio (LMR); stem mass ratio (SMR); root mass ratio (RMR); dead leaf biomass ratio (DLR).

% Change						
		GLBP	LMR	SMR	RMR	DMR
CIAT 679	Cylinder (4kg)	101.4	85.5	113.7	93.2	74.7
CIAT 26110	Cylinder (4kg)	88.1	121.2	125.5	19.0	448.3
Br 44-02	Cylinder (4kg)	58.7	72.5	183.9	25.9	455.0
CIAT 679	Pot (3.5 kg)	107.9	107.4	108.1	84.2	60.7
CIAT 26110	Pot (3.5 kg)	89.8	104.0	84.7	103.2	198.7
Br 44-02	Pot (3.5 kg)	70.8	52.9	122.4	109.6	324.0
CIAT 679	Pot (1.75kg)	99.4	108.5	114.5	73.7	109.9
CIAT 26110	Pot (1.75kg)	82.7	65.7	110.9	117.3	214.7
Br 44-02	Pot (1.75kg)	76.7	64.0	103.8	113.6	205.5

Conclusions

Waterlogging induced a reduction in biomass in the genotypes tested but size of container influenced the response to waterlogging. More contrasting differences under waterlogged conditions among genotypes were found using cylinders filled with 4Kg of soil.

Acknowledgements

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